

WHAT IS CLAIMED IS:

1 1 A polypeptide comprising a mutated antibody heavy chain variable
 2 region or light chain variable region, the polypeptide having at least 5 times higher
 3 binding affinity for an antigen than does a parental antibody, the polypeptide having a
 4 sequence that differs from the parental antibody by an amino acid substitution of at least
 5 one amino acid in a complementarity determining region (CDR), the amino acid encoded
 6 by a codon that comprises a nucleotide belonging to a hot spot motif selected from AGY
 7 or RGYW, wherein R is A or G, Y is C or T and W is A or T.

112 2nd
 5'05T
 0-2
 0-2
 NNS
 S-G-0-
 C

1 2. The polypeptide of claim 1 wherein the substitution occurs in
 2 CDR3 of a light chain variable region.

1 3. The polypeptide of claim 1 wherein the substitution occurs in
 2 CDR3 of a heavy chain variable region.

1 4. The polypeptide of claim 1 wherein the substitution occurs in
 2 CDR1 or CDR2 of a light chain variable region.

1 5. The polypeptide of claim 1 wherein the substitution occurs in
 2 CDR1 or CDR2 of a heavy chain variable region.

1 6. The polypeptide of claim 2 wherein the antigen is mesothelin, the
 2 parental antibody is antimesothelin antibody SS and the polypeptide has a sequence that
 3 differs from antibody SS by an amino acid substitution of at least one amino acid selected
 4 from S92, G93 and Y94.

DEPOSIT

1 7. The polypeptide of claim 6 wherein the substitutions are selected
 2 from G93K-Y94H (SS1); S92G-G93F-Y94N (D8) and S92G-G93S-Y94H (C10).

1 8. The polypeptide of claim 1, wherein said polypeptide is a scFv.

1 9. The polypeptide of claim 6, wherein said polypeptide is a scFv.

1 10. The polypeptide of claim 1, wherein said polypeptide is a dsFv, a
 2 Fab, or a F(ab')₂.

- 1 11. The polypeptide of claim 6, wherein said polypeptide is a dsFv, a
2 Fab, or a F(ab')₂.
- 1 12. The polypeptide of claim 1 further comprising a therapeutic moiety
2 or a detectable label.
- 1 13. The polypeptide of claim 12, wherein the therapeutic moiety is a
2 toxic moiety.
- 1 14. The polypeptide of claim 13, wherein the toxic moiety is a
2 *Pseudomonas* exotoxin or a cytotoxic fragment thereof.
- 1 15. The polypeptide of claim 14, wherein the toxic moiety is a
2 cytotoxic fragment, which is PE38.
- 1 16. The polypeptide of claim 13, wherein the toxic moiety is selected
2 from the group consisting of diphtheria toxin or a cytotoxic fragment thereof, saporin or a
3 cytotoxic fragment thereof, pokeweed antiviral toxin or a cytotoxic fragment thereof,
4 ricin or a cytotoxic fragment thereof, and bryodin 1 or a cytotoxic fragment thereof.
- 1 17. The polypeptide of claim 6, further comprising a therapeutic
2 moiety or a detectable label
- 1 18. The polypeptide of claim 17, wherein the therapeutic moiety is a
2 toxic moiety.
- 1 19. The polypeptide of claim 18, wherein the toxic moiety is a
2 *Pseudomonas* exotoxin or a cytotoxic fragment thereof.
- 1 20. The polypeptide of claim 19, wherein the toxic moiety is a
2 cytotoxic fragment, selected from the group consisting of PE35, PE38, and PE40.
- 1 21. The polypeptide of claim 1, further comprising a surface protein of
2 a bacteriophage.
- 1 22. A polypeptide which has a binding affinity for mesothelin at least
2 three times that of antimesothelin antibody SS, which polypeptide has a sequence which

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3 differed from antibody SS by an amino acid substitution in CDR3 of a light chain variable
4 region of at least L96T (E4).

1 23. The polypeptide of claim 22, further comprising a therapeutic
2 moiety or a detectable label.

1 24. The polypeptide of claim 23, wherein the therapeutic moiety is a
2 toxic moiety.

1 25. The polypeptide of claim 24, wherein the toxic moiety is a
2 *Pseudomonas* exotoxin or a cytotoxic fragment thereof.

1 26. The polypeptide of claim 25, wherein the toxic moiety is a
2 cytotoxic fragment, selected from the group consisting of PE35, PE38, and PE40.

1 27. A nucleic acid molecule encoding a polypeptide comprising a
2 mutated antibody heavy chain variable region or light chain variable region, the
3 polypeptide having at least 5 times higher binding affinity for an antigen than does a
4 parental antibody, the polypeptide having a sequence that differs from a parental antibody
5 by an amino acid substitution of at least one amino acid in a complementarity determining
6 region (CDR), the amino acid encoded by a codon that comprises a nucleotide belonging
7 to a hot spot motif selected from AGY or RGYW, wherein R is A or G, Y is C or T and
8 W is A or T.

1 28. The nucleic acid molecule of claim 27, wherein the antigen is
2 mesothelin, the parental antibody is antimesothelin antibody SS and the polypeptide has a
3 sequence that differs from antibody SS by an amino acid substitution of at least one
4 amino acid selected from S92, G93 and Y94.

1 29. The nucleic acid molecule of claim 27, wherein the substitutions
2 are selected from G93K-Y94H (SS1); S92G-G93F-Y94N (D8) and S92G-G93S-Y94H
3 (C10).

1 30. A nucleic acid molecule encoding a polypeptide which has a
2 binding affinity for mesothelin at least three times that of antimesothelin antibody SS,
3 which polypeptide has a sequence which differed from antibody SS by an amino acid
4 substitution in CDR3 of a light chain variable region of at least L96T (E4).

1 31. An expression cassette comprising a promoter operably linked to a
2 nucleic acid molecule of claim 27.

1 32. An expression cassette comprising a promoter operably linked to a
2 nucleic acid molecule of claim 28.

1 33. A method of killing a malignant cell bearing an antigen,
2 comprising contacting the cell with an immunotoxin comprising a toxic moiety and a
3 targeting moiety, the targeting moiety comprising a polypeptide comprising a mutated
4 antibody heavy chain variable region or light chain variable region, the polypeptide
5 having at least 5 times higher binding affinity for an antigen than does a parental
6 antibody, the polypeptide having a sequence that differs from the parental antibody by an
7 amino acid substitution of at least one amino acid in a complementarity determining
8 region (CDR), the amino acid encoded by a codon that comprises a nucleotide belonging
9 to a hot spot motif selected from AGY or RGYW, wherein R is A or G, Y is C or T and
10 W is A or T.

1 34. The method of claim 33, wherein the antigen is mesothelin.

1 35. The method of claim 34, wherein the targeting moiety is selected
2 from the group consisting of SS1, D8, and C10.

1 36. The method of claim 35, wherein said toxic moiety is a
2 *Pseudomonas* exotoxin or cytotoxic fragment thereof.

1 37. The method of claim 36, wherein the toxic moiety is a cytotoxic
2 fragment selected from the group consisting of PE35, PE38, and PE40.

1 38. A method of killing a malignant cell bearing an antigen,
2 comprising contacting the cell with an immunotoxin comprising a toxic moiety and a
3 targeting moiety, wherein the targeting moiety is antibody E4.

1 39. The method of claim 38, wherein said toxic moiety is a
2 *Pseudomonas* exotoxin or cytotoxic fragment thereof.

1 40. The method of claim 38, wherein the toxic moiety is a cytotoxic
2 fragment selected from the group consisting of PE35, PE38, and PE40

1 41. A method of identifying a polypeptide which has a higher affinity
2 for a target antigen than does a parental antibody, comprising

3 (a) contacting a polypeptide of claim 1 with the target antigen under
4 conditions appropriate for specific binding between an antibody and the target antigen,

5 (b) eluting the polypeptide under conditions which remove any
6 antibody or fragment thereof which have not bound to the target antigen with an affinity
7 higher than that of the parental antibody or fragment thereof, and

8 (c) determining whether the polypeptide is bound to the antigen,
9 whereby binding identifies the polypeptide as having a higher affinity for the target than
10 does the parental antibody.

1 42. A method of making a library of nucleic acids encoding mutated
2 antibody variable domains comprising:

3 a) providing a nucleic acid molecule encoding an amino acid
4 sequence of a V_H or a V_L domain of a parental antibody, the nucleic acid molecule
5 comprising at least one parental hot spot codon comprising at least one nucleotide within
6 a hot spot motif;

7 b) generating a plurality of mutated nucleic acid molecules encoding
8 mutated amino acid sequences that differ from the parental amino acid sequence wherein
9 each mutated nucleic acid sequence comprises at least one mutated codon different than a
10 parental hot spot codon encoding an amino acid, the mutated codon encoding an amino
11 acid different than the amino acid encoded by the parental hot spot codon.

1 43. The method of claim 42 wherein the plurality of mutated nucleic
2 acid molecules contains at least 19 members, wherein each of the 19 members encodes an
3 amino acid sequence in which the amino acid encoded by the parental hot spot codon is
4 replaced by a different natural amino acid.

1 44. The method of claim 42, wherein the plurality of mutated nucleic
2 acid molecules comprises mutated codons different than at least two parental hot spot
3 codons encoding amino acids, each of the mutated codons encoding an amino acid
4 different than the amino acid encoded by the parental hot spot codon.

1 45. The method of claim 42, wherein the plurality of mutated nucleic
2 acid molecules comprises at least 399 members, each of which members encodes an

3 amino acid sequence in which the amino acids encoded by the parental hot spot codons is
4 replaced by a different natural amino acid.

1 46. The method of claim 42, further wherein the parental antibody is of
2 a class of antibodies having at least one conserved amino acid encoded by a codon,
3 wherein the codon or codons encoding the conserved amino acids are not mutated.

1 47. The method of claim 42, wherein the hot spot motif is selected
2 from the group consisting of AGCA, AGTT, AGCT, AGTA, GGCA, GGTT, GGCT,
3 GGTA, AGC, and AGT.

1 48. The method of claim 42, wherein the mutated nucleic acid
2 molecule comprises at least one mutated codon within a portion of the V_H or the V_L
3 domain comprising a CDR.

1 49. The method of claim 48, wherein the CDR is the CDR3 of the V_H
2 domain.

1 50. The method of claim 48, wherein the CDR is the CDR3 of the V_L
2 domain.